EXHIBIT D

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Boyle et al.

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For: Osteoprotegerin

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Considered 11/7/00 GW

DECLARATION OF DR. JACKIE Z. SHENG

I, Jackie Z. Sheng, declare and state as follows:

1. Since 1994, I have been employed by Amgen, Inc., Thousand Oaks, CA and currently hold the position of Research Scientist.

2. From 1992 to 1994 I was Assistant Instructor in the Department of Molecular Genetics at the University of Texas Southwestern Medical School, Dallas, TX. From 1990 to 1992 I was a postdoctoral associate in the Department of Biochemistry at Purdue University, West Lafayette, IN. My research interests are in the areas of gene therapy, molecular virology and molecular biology. Submitted herewith as Attachment No. 1 is a copy of my cirriculum vitae.

3. Since joining Amgen, I have been involved in experiments designed to test various viral vectors to deliver polypeptides for gene therapy applications.

4. I conducted experiments designed to test the activity of OPG upon gene transfer into mice. In experiments that were performed under my direction, I constructed a recombinant adenovirus-based vector for delivery of an OPG fusion polypeptide. The OPG fusion polypeptide comprised amino acids 1-201 of OPG (hereafter OPG[1-201]) as shown in Figure 9B (SEQ ID NO: 5) of the present application, U.S. Serial No. 08/974,186, fused at its carboxy terminus to the hinge, CH2 and CH3 regions of human IgGγ1 as shown in Ellison et al. (Nuc. Acids Res. 10, 4071-4079 (1982) submitted as Attachment No. 2). DNA encoding the OPG[1-201]-Fc fusion polypeptide was inserted into the

adenoviral shuttle plasmid pACCMVpLpA (described in Gomez-Foiz et al. J. Biol. Chem. 267, 25129-25134 (1992) submitted as Attachment No. 3) as follows. pACCMVpLpA was digested with BamHI, treated with alkaline phosphatase, and digested with SalI. OPG[1-201]-Fc DNA in plasmid pDSRα2 (pDSRα2 is described in WO90/14363) was digested with BamHI and SalI and the DNA fragments ligated. The resulting recombinant plasmid is designated pACCMV-OPG[1-201]-Fc. pACCMV OPG[1-201]-Fc and the large fragment of XbaI-cut plasmid RR5 (RR5 is an Ad-dL309 derivative described in Kopfler et al. Circulation 90, 1319-1327 (1994) submitted as Attachment No. 4) were cotransfected into 293 human embryonic kidney cells (available from the American Type Culture Collection, Manassas, VA under accession no. CRL-1573). Transfected 293 cells were overlaid with DMEM containing 0.65% agarose, 2% fetal calf serum supplemented with penicillin and streptomycin. Recombinant viral plaques containing OPG[1-201]-Fc DNA were isolated, subjected to an additional round of plaque purification, and expanded into stocks containing 5 x 10^{10} plaque forming units (pfu)/ml, using procedures generally described in Attachment No. 4. Recombinant adenovirus were titered by a plaque assay on 293 cells using procedures described in Attachment No. 4.

5. I confirm that the following reagents: OPG DNA, human IgGγ1 DNA, plasmid pACCMVpLpA, plasmid RR5, and 293 human embryonic kidney cell line were available to one skilled in the art before the priority date of the present application.

6. Based upon information provided to me, ovariectomized and sham-operated mice were prepared as follows. Female C57/BL6 mice aged 8 weeks (Charles River, Wilmington, MA) were anesthetized with isofluorane, shaved and scrubbed with an iodophore solution. An incision was made on one side of the animal and muscle was dissected until fat surrounding the uterus and ovary was exposed. The ovary and uterus were pulled out of the incision and the ovary was clamped at the uterus and removed. The uterus was placed back into the body cavity and the incision closed with skin clips. The same procedure was repeated on the other side of the animals. For sham operated controls, the ovary and uterus were pulled from the incision but the ovary was not removed.

7. In experiments done under my direction, each group of ovariectomized and sham-operated mice (eight animals per group) were injected into the tail vein with one of the

following: about 5 x 10^8 pfu of recombinant adenovirus in PBS carrying OPG[1-201]-Fc DNA; about 5 x 10^8 recombinant adenovirus carrying cDNA encoding β-galactosidase (referred to as AdCMV-βgal in Herz et al. (Proc. Natl. Acad. Sci. USA 90, 2812-2816 (1993), submitted as Attachment No. 5); and a vehicle control of PBS with no virus. 28 days after injection, the mice were euthanized with carbon dioxide.

8. Based upon information provided to me, bone mineral density at two sites, the proximal tibial metaphysis and the lumbar vertebrae (L5), were determined as follows. The fully articulated tibia and femur were removed from the euthanized mice and fixed in 70% ethanol. Bone mineral density was determined at the proximal tibial metaphysis by peripheral quantitative computed tomography (pQCT) (XCT-960M, Norland Medical Systems, Ft Atkinson, WI). Two 0.5mm cross-sections of bone, 1.5mm and 2.0mm from the proximal end of the tibia were analyzed (XMICE 5.2, Stratec, Germany) to determine total and trabecular (defined as the inner most 20% of the bone cross-section) bone mineral density in the metaphysis, and an average value for both cross-sections is reported. A soft tissue separation threshold of 1500 was used to define the boundary of the metaphyseal bone. The total bone mineral density for lumbar vertabrae (L5) was determined in a similar manner. Fully articulated sections of lumbar vertebrae were extracted from the mice and L5 was removed and fixed in 70% ethanol. L5 is designated as the first vertebrae proximal to the iliac crest. A single 1.0mm mid-vertebral cross-section of bone was analyzed. A soft tissue separation threshold of 1500 was used.

9. Based upon information provided to me, the measurements of bone mineral density at the tibial metaphysis and in lumbar vertebrae in ovariectomized and sham-operated mice are shown in Attachment No. 6 (for the tibial metaphysis) and Attachment No. 7 (for the lumbar vertebrae). Ovariectomized mice treated with vehicle (PBS with no virus) had significantly lower bone mineral density at both sites compared to sham-operated mice treated in the same manner. Injection of pACCMV OPG[1-201]-Fc vector into ovarectomized mice increased bone mineral density at both sites compared to sham operated control mice and blocked the loss of bone density in these mice. Injection of the control vector (AdCMV-βgal) did not affect bone